

REVIEWED

By Celena Shrum at 3:41 pm, Dec 08, 2021

SC

12/8/2021

Worklist: 5431

<u>LAB_CASE</u>	<u>ITEM</u>	<u>ITEM_TYPE</u>	<u>DESCRIPTION</u>
M2020-0952	16	BLOOD	AM 28 Blood Multi-Drug Quant Panel 1 by LC-QQQ



SC

AM# 28: Multi-Drug Quantitation in Blood by LC-MS/MS

Extraction Date: 12/08/21

Analyst: Sarah Collins

Plate lot#: IDP-121-210610

Retest Date: 12/10/2021

Mobile phase A: 5mM Amm Form + 0.01% FA
0.5M Ammonium Hydroxide

Mobile phase B: 0.01% Formic Acid in MeOH
Ethyl Acetate 20% Methanol in Water

Blank Blood Lot: Lampire ~~20L20724~~ 20L20725

Column: Agilent 120 EC-C18 (2.1x 100-2.7um)

LCMS-QQQ ID: 069901 12/8/21 SC

Pre-Analytic:

- 1. Check levels of mobile phases and needle wash refill as needed. Ensure waste is not full.
- 2. Ensure correct column is installed and begin mobile phase flow allow to equilibrate ~ 30 minutes.

Analytic:

- 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
- 2. Pipette **250 µL blood (calibrated pipette)** in wells of analytical (standards) plate. **Pipette ID: #16**
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 4. Pipette **250 µL 0.5 M ammonium hydroxide** in wells of analytical plate.
- 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 6. Transfer **300 µL of blood+base** mixture to corresponding wells of SLE+ plate.
- 7. Apply positive pressure for approx. 10-15 seconds (or until no liquid remains on top of sorbent).
(Load at 85-100 PSI- Selector to the right)
- 8. Wait 5 minutes.
- 9. Add **900 µL ethyl acetate.**
- 10. Wait 5 minutes.
- 11. Apply positive pressure for approx. 10-15 seconds. *(12-15 PSI- Selector to the left).*
- 12. Add **900 µL ethyl acetate.**
- 13. Wait 5 minutes.
- 14. Apply positive pressure for approx. 15 seconds. *(12-15 PSI- Selector to the left).*
- 15. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C.
- 16. Reconstitute in **100 µL 20% LCMS MeOH in water** and heat seal plate with foil. Place in autosampler and run worklist.

Post-Analytic

- 1. Create batch and process data.
- 2. Make necessary changes to integration limits
- 3. Integration linear and R² values ≥0.98 for each analyte.
- 4. For unknown samples and controls: response ratio within 20% of average of controls and standards, RT within +/- 5% (tramadol RT +/-2%), S/N for primary transition >10 and secondary transitions >5.
- 5. Did all QCs pass for each analyte? Y / N _____ Add Control data to QC tracking spreadsheet.
- 6. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports.

COMMENTS:

Only alpha-hydroxyalprazolam and alprazolam were evaluated in this run.

Alpha-hydroxyalprazolam 5-250

Alprazolam-D5 used as alpha-hydroxyalprazolam ISTD and thus reported qualitatively

Analytical Plate Map

SC

	1	2	3	4	5	6	7	8	9	10	11	12
A	IS + Cal. 1	IS + QC_1			IS + Cal. 1	IS + QC_1						IS + Cal. 8
B	IS + Cal. 2	IS + QC_2			IS + Cal. 2	IS + QC_2						IS + Cal. 7
C	IS + Cal. 3	IS + QC_3			IS + Cal. 3	IS + QC_3						IS + Cal. 6
D	IS + Cal. 4	IS + QC_4			IS + Cal. 4	IS + QC_4					IS + QC_2 urine	IS + Cal. 5
E	IS + Cal. 5	IS + QC_2			IS + Cal. 5	IS + QC_2 urine					IS + QC_4	IS + Cal. 4
F	IS + Cal. 6				IS + Cal. 6	negative blood					IS + QC_3	IS + Cal. 3
G	IS + Cal. 7				IS + Cal. 7	m2020-0952-1					IS + QC_2	IS + Cal. 2
H	IS + Cal. 8				IS + Cal. 8						IS + QC_1	IS + Cal. 1

All wells to contain 60 µl of Trapping Solution

SLE Plate Map

SC

	1	2	3	4	5	6	7	8	9	10	11	12
A											IS + Cal. 1	IS + QC_1
B											IS + Cal. 2	IS + QC_2
C											IS + Cal. 3	IS + QC_3
D											IS + Cal. 4	IS + QC_4
E											IS + Cal. 5	IS + QC_2 urine
F											IS + Cal. 6	negative blood
G											IS + Cal. 7	m2020-0952- 1
H											IS + Cal. 8	

All wells to contain 60 µl of Trapping Solution

SC

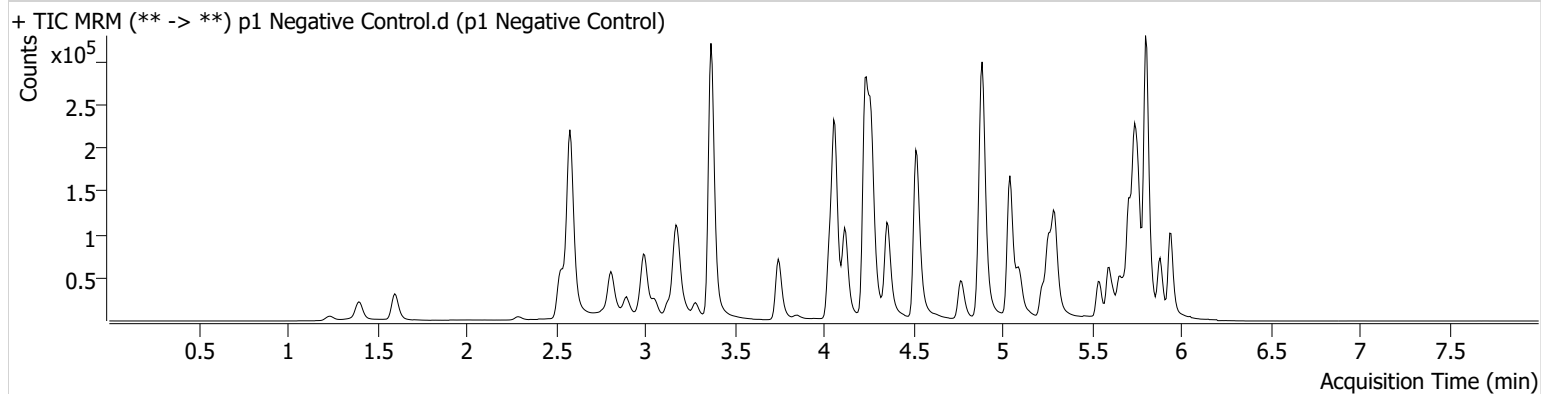


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12082021 AM 28 SC\QuantResults\AM 28 casework.batch.bin
Calibration Last Update 12/8/2021 2:36:22 PM

Instrument	Falco (069901)	Data File	p1 Negative Control.d
Type	Sample	Sample	p1 Negative Control
Acq. Method	AM 28 MDQ P1.m	Operator	Sarah Collins
Sample Position	P2-F12	Comment	
Injection Volume	2		
Acq. Date-Time	12/8/2021 12:23:13 PM		
Sample Info.			

Sample Chromatogram



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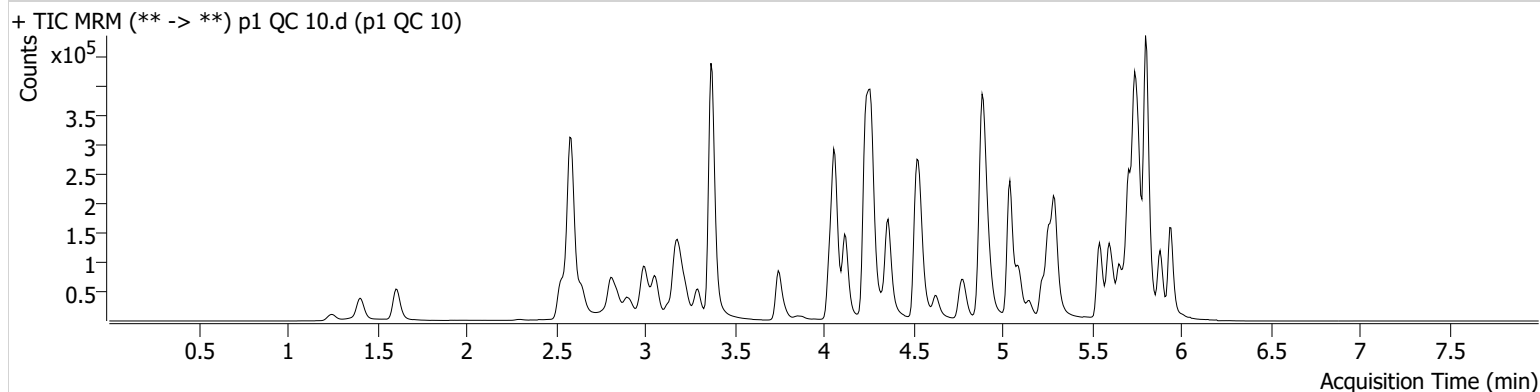


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12082021 AM 28 SC\QuantResults\AM 28 casework.batch.bin
Calibration Last Update 12/8/2021 2:36:22 PM

Instrument	Falco (069901)	Data File	p1 QC 10.d
Type	QC	Sample	p1 QC 10
Acq. Method	AM 28 MDQ P1.m	Operator	Sarah Collins
Sample Position	P2-A12	Comment	
Injection Volume	2		
Acq. Date-Time	12/8/2021 11:08:54 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
a-hydroxyalprazolam	5.685	20967	3617.83	71.9	140.91	247652	9.6583 ng/ml
Alprazolam	5.768	76885	548.86	111.9	344.08	247652	10.2685 ng/ml

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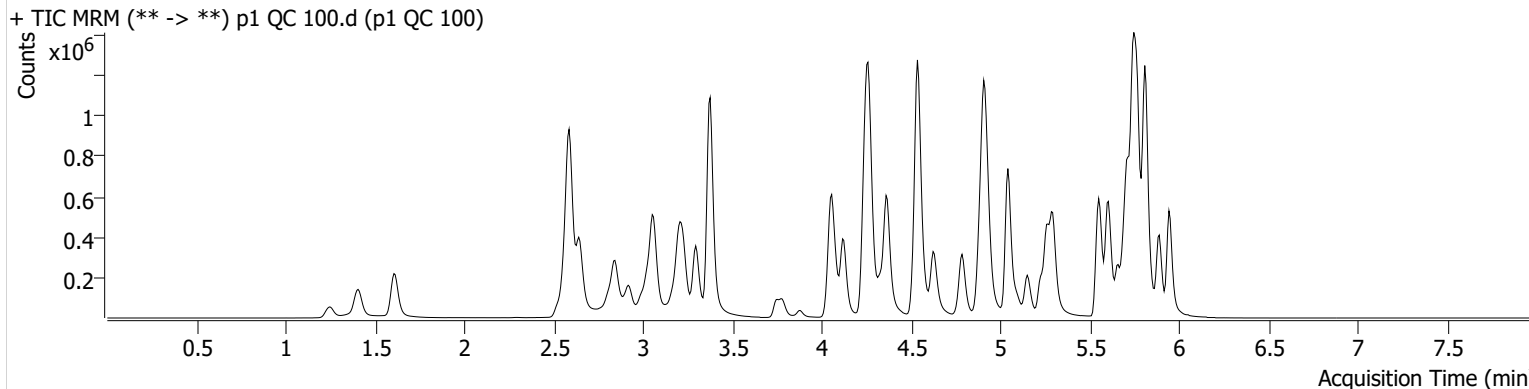


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12082021 AM 28 SC\QuantResults\AM 28 casework.batch.bin
Calibration Last Update 12/8/2021 2:36:22 PM

Instrument	Falco (069901)	Data File	p1 QC 100.d
Type	QC	Sample	p1 QC 100
Acq. Method	AM 28 MDQ P1.m	Operator	Sarah Collins
Sample Position	P2-B12	Comment	
Injection Volume	2		
Acq. Date-Time	12/8/2021 1:05:40 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
a-hydroxyalprazolam	5.685	170058	294.23	72.7	334.37	208838	105.0833 ng/ml
Alprazolam	5.768	687460	2195.07	110.7	1380.20	208838	112.0551 ng/ml

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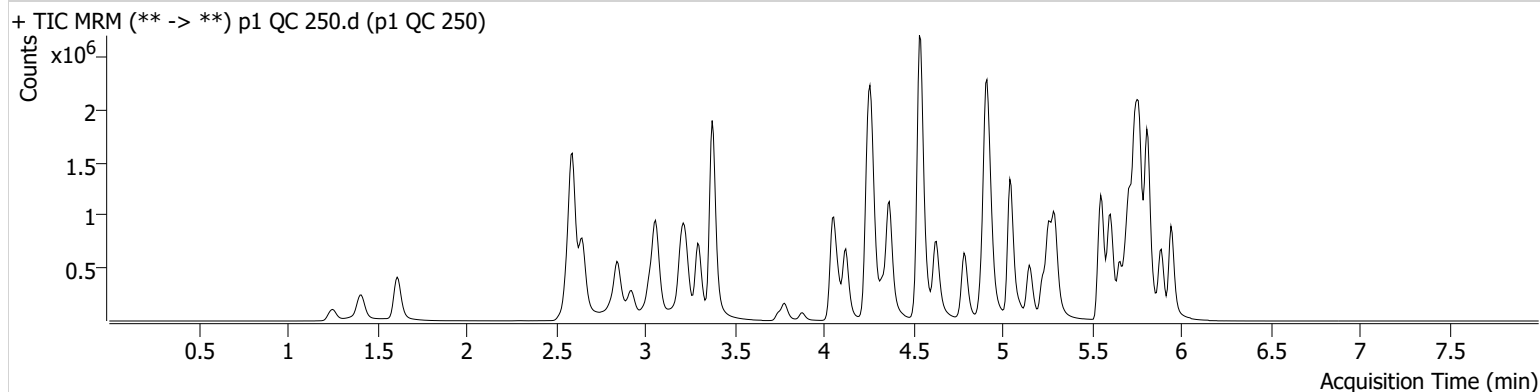


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12082021 AM 28 SC\QuantResults\AM 28 casework.batch.bin
Calibration Last Update 12/8/2021 2:36:22 PM

Instrument	Falco (069901)	Data File	p1 QC 250.d
Type	QC	Sample	p1 QC 250
Acq. Method	AM 28 MDQ P1.m	Operator	Sarah Collins
Sample Position	P2-C12	Comment	
Injection Volume	2		
Acq. Date-Time	12/8/2021 11:30:08 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
a-hydroxyalprazolam	5.685	271556	1979.45	75.1	326.48	165076	213.7285 ng/ml
Alprazolam	5.768	1365577	1191.65	107.4	1330.83	165076	282.0956 ng/ml

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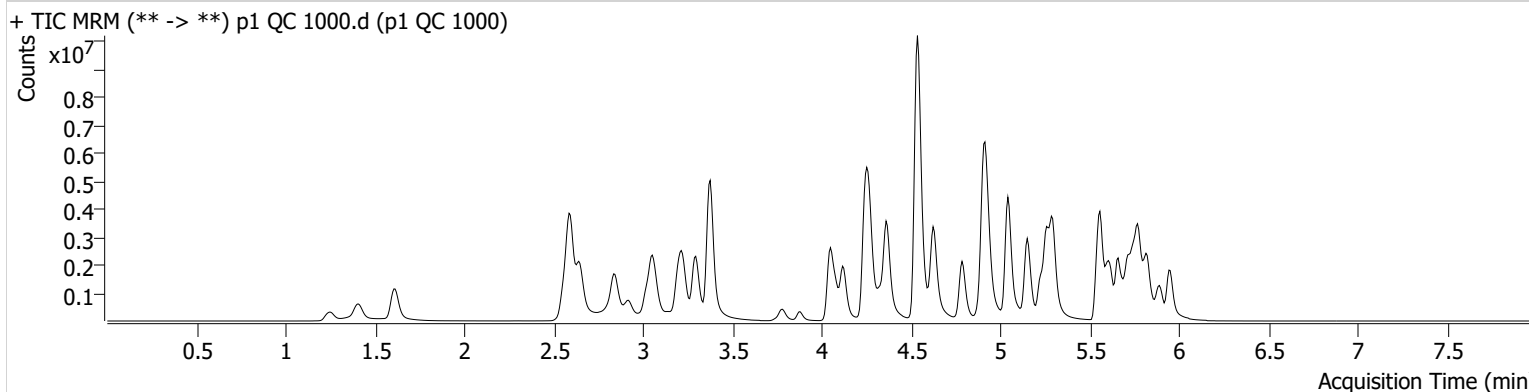


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12082021 AM 28 SC\QuantResults\AM 28 casework.batch.bin
Calibration Last Update 12/8/2021 2:36:22 PM

Instrument	Falco (069901)	Data File	p1 QC 1000.d
Type	QC	Sample	p1 QC 1000
Acq. Method	AM 28 MDQ P1.m	Operator	Sarah Collins
Sample Position	P2-D12	Comment	
Injection Volume	2		
Acq. Date-Time	12/8/2021 11:51:22 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
* α-hydroxyalprazolam	5.685	411458	569.38	71.9	3887.57	99563	539.0642 ng/ml
Alprazolam	5.768	3209915	1012.82	108.0	755.50	99563	1100.3661 ng/ml

*OCR

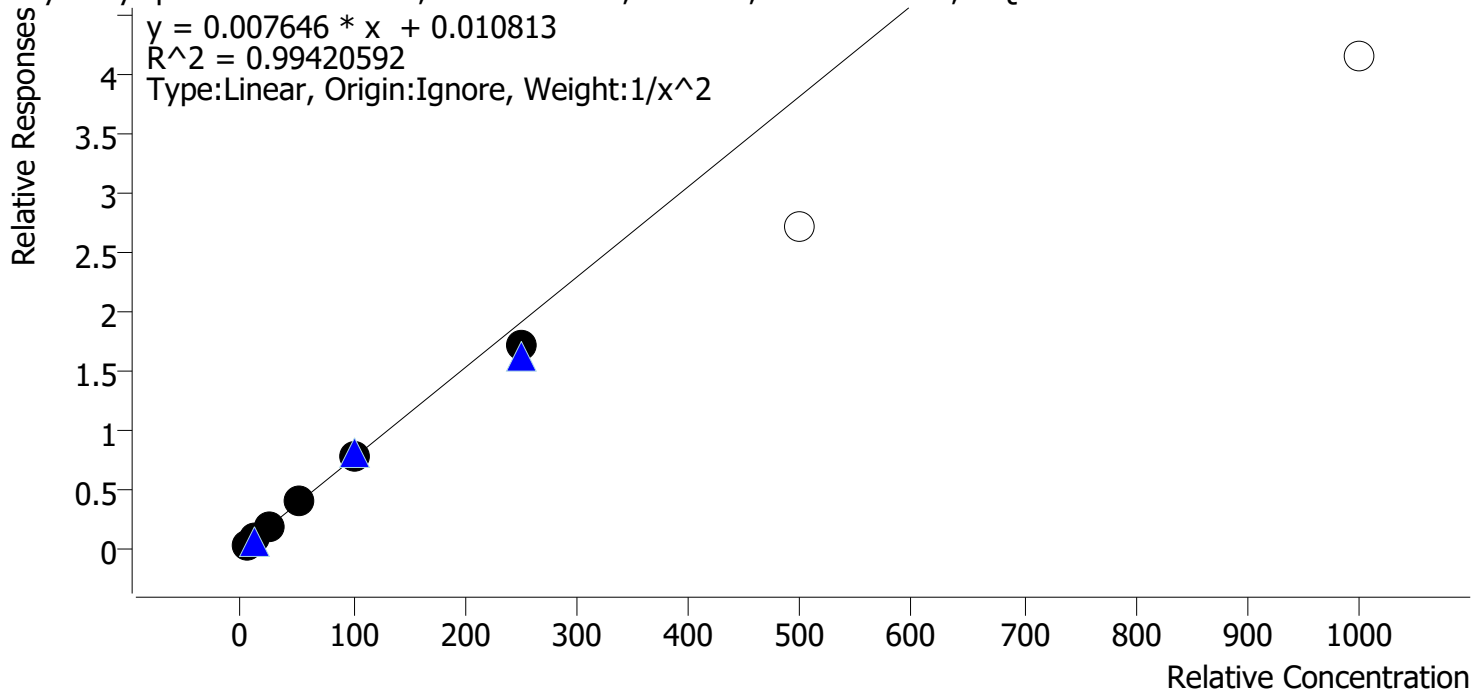
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AM #28 Multi-Drug Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\12082021 AM 28 SC\QuantResults\AM 28 casework.batch.bin
Last Cal. Update 12/8/2021 2:36 PM
Analyst Name ISP\datastor
Analyte a-hydroxyalprazolam **Internal Standard** Alprazolam-D5

a-hydroxyalprazolam - 8 Levels, 6 Levels Used, 8 Points, 6 Points Used, 4 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p1 Cal 1-5ng	1	✓	5.0	4.8	96.1
p1 Cal 2-10ng	2	✓	10.0	10.6	106.3
p1 Cal 3 -25ng	3	✓	25.0	25.5	102.1
p1 Cal 4-50ng	4	✓	50.0	52.7	105.4
p1 Cal 5-100ng	5	✓	100.0	100.4	100.4
p1 Cal 6-250ng	6	✓	250.0	224.5	89.8
p1 Cal 7-500ng	7	x	500.0	355.2	71.0
p1 Cal 8-1000ng	8	x	1000.0	543.2	54.3

Calibrators 7 and 8 dropped due to outside accuracy range

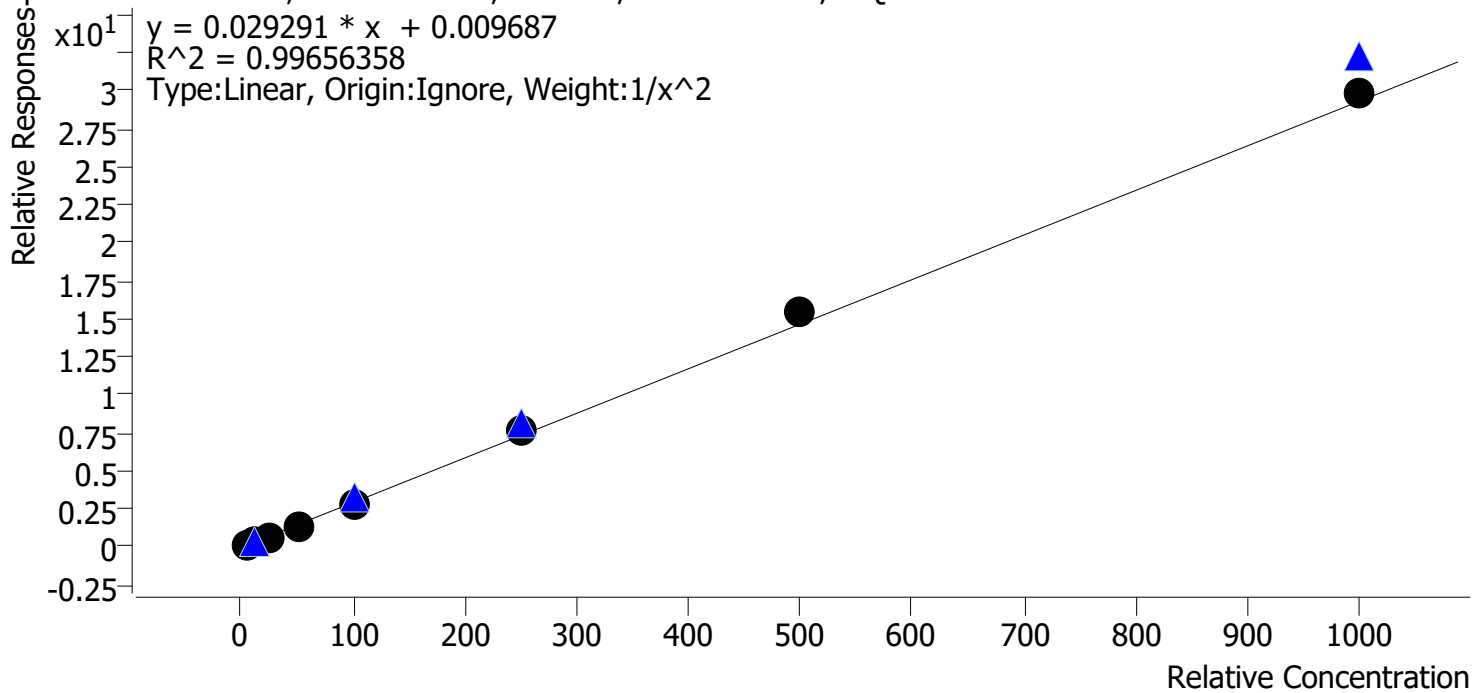
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AM #28 Multi-Drug Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\12082021 AM 28 SC\QuantResults\AM 28 casework.batch.bin
Last Cal. Update 12/8/2021 2:36 PM
Analyst Name ISP\datastor
Analyte Alprazolam **Internal Standard** Alprazolam-D5

Alprazolam - 8 Levels, 8 Levels Used, 8 Points, 8 Points Used, 4 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p1 Cal 1-5ng	1	✓	5.0	5.2	103.2
p1 Cal 2-10ng	2	✓	10.0	9.8	97.6
p1 Cal 3 -25ng	3	✓	25.0	23.0	91.9
p1 Cal 4-50ng	4	✓	50.0	47.8	95.5
p1 Cal 5-100ng	5	✓	100.0	97.9	97.9
p1 Cal 6-250ng	6	✓	250.0	263.9	105.6
p1 Cal 7-500ng	7	✓	500.0	531.6	106.3
p1 Cal 8-1000ng	8	✓	1000.0	1020.7	102.1

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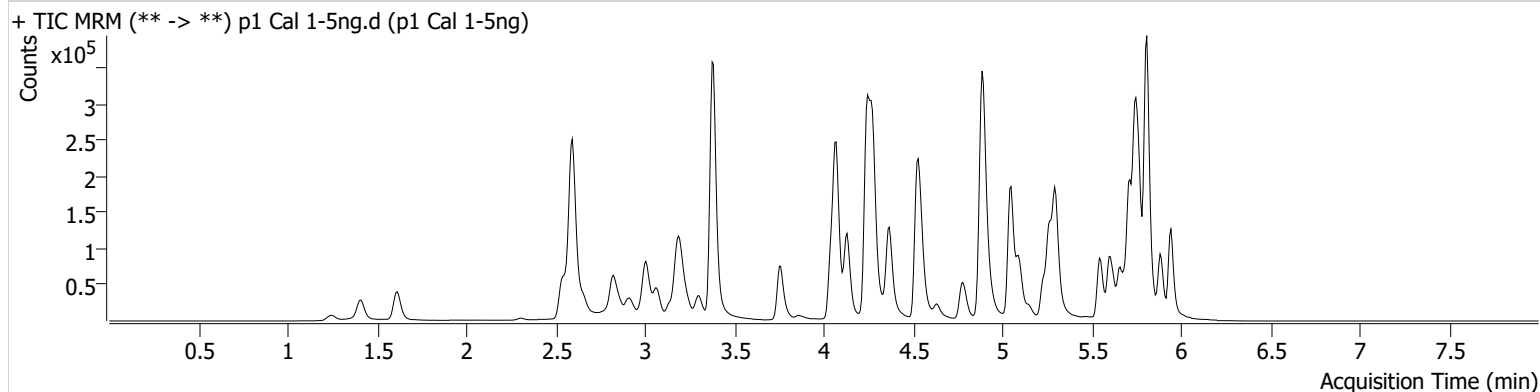


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12082021 AM 28 SC\QuantResults\AM 28 casework.batch.bin
Calibration Last Update 12/8/2021 2:36:22 PM

Instrument	Falco (069901)	Data File	p1 Cal 1-5ng.d
Type	Cal	Sample	p1 Cal 1-5ng
Acq. Method	AM 28 MDQ P1.m	Operator	Sarah Collins
Sample Position	P2-A11	Comment	
Injection Volume	2		
Acq. Date-Time	12/8/2021 9:22:34 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
a-hydroxyalprazolam	5.692	10238	112.98	76.2	217.32	215344	4.8036 ng/ml
Alprazolam	5.768	34634	157.16	114.5	283.62	215344	5.1602 ng/ml

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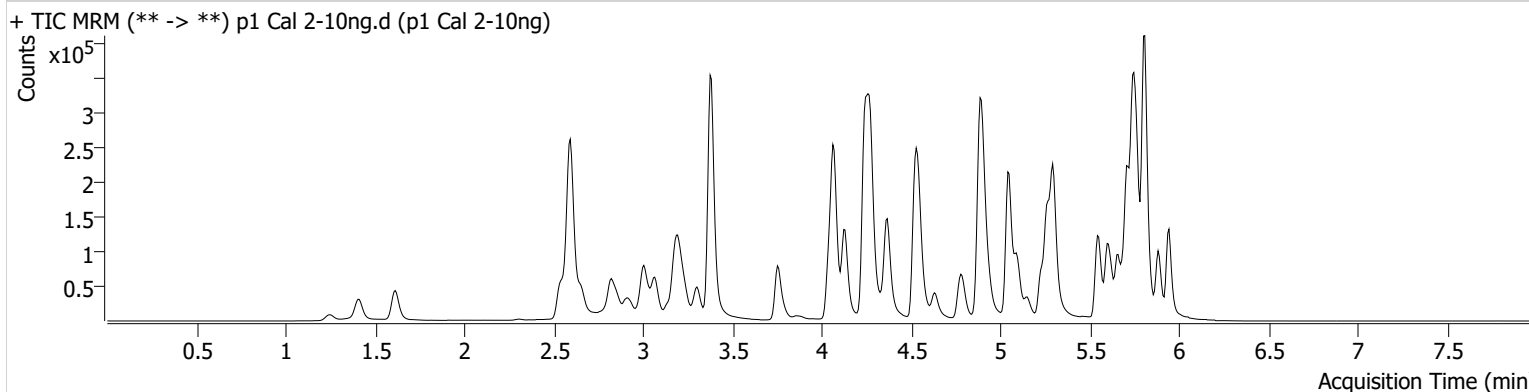


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12082021 AM 28 SC\QuantResults\AM 28 casework.batch.bin
Calibration Last Update 12/8/2021 2:36:22 PM

Instrument	Falco (069901)	Data File	p1 Cal 2-10ng.d
Type	Cal	Sample	p1 Cal 2-10ng
Acq. Method	AM 28 MDQ P1.m	Operator	Sarah Collins
Sample Position	P2-B11	Comment	
Injection Volume	2		
Acq. Date-Time	12/8/2021 9:33:21 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
a-hydroxyalprazolam	5.692	19130	415.60	71.4	415.13	207681	10.6327 ng/ml
Alprazolam	5.768	61372	499.51	111.1	348.82	207681	9.7583 ng/ml

SC

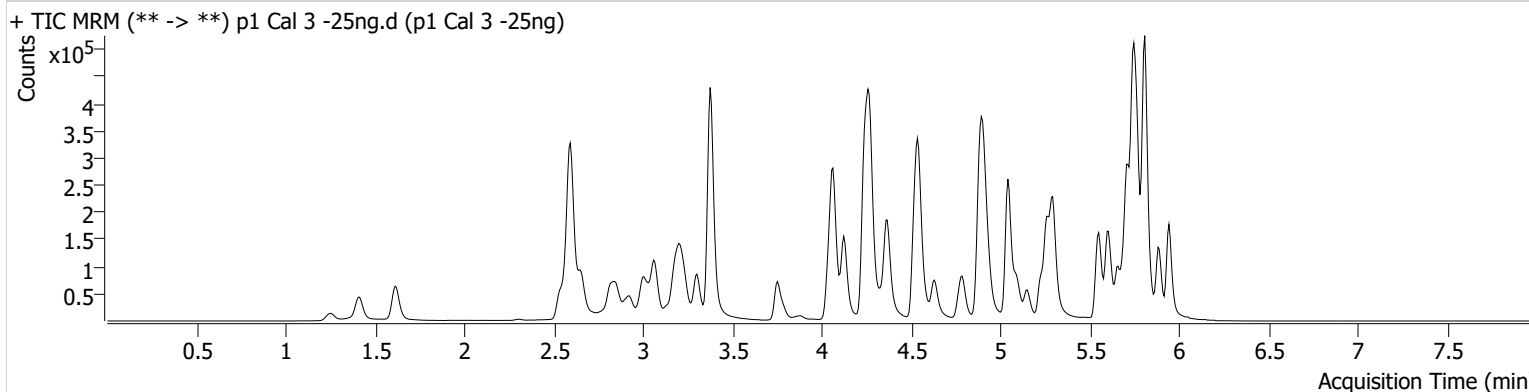


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12082021 AM 28 SC\QuantResults\AM 28 casework.batch.bin
Calibration Last Update 12/8/2021 2:36:22 PM

Instrument	Falco (069901)	Data File	p1 Cal 3 -25ng.d
Type	Cal	Sample	p1 Cal 3 -25ng
Acq. Method	AM 28 MDQ P1.m	Operator	Sarah Collins
Sample Position	P2-C11	Comment	
Injection Volume	2		
Acq. Date-Time	12/8/2021 9:43:57 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
a-hydroxyalprazolam	5.692	40581	148.84	74.7	468.61	197092	25.5137 ng/ml
Alprazolam	5.768	134494	356.03	114.9	513.05	197092	22.9666 ng/ml

SC

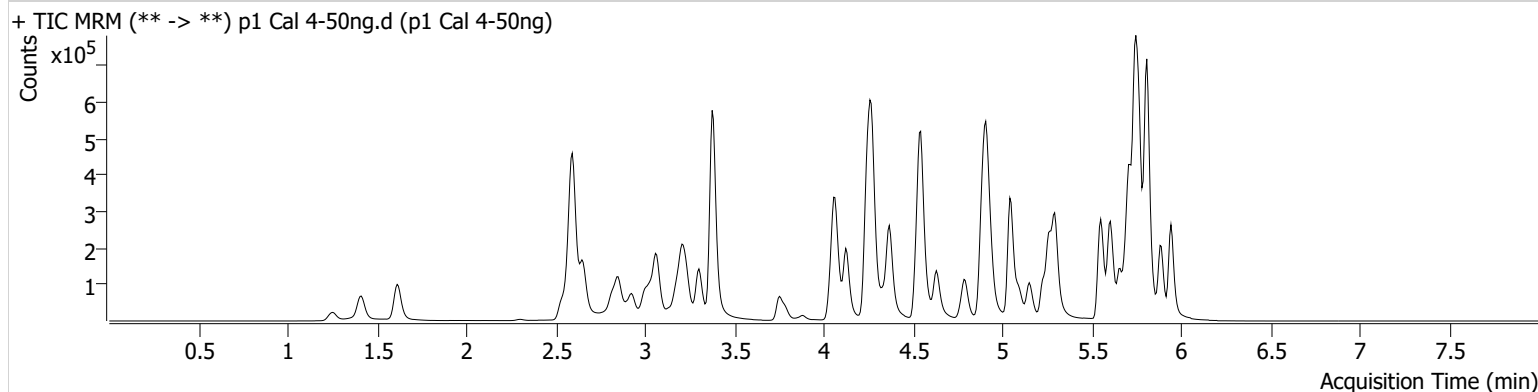


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12082021 AM 28 SC\QuantResults\AM 28 casework.batch.bin
Calibration Last Update 12/8/2021 2:36:22 PM

Instrument	Falco (069901)	Data File	p1 Cal 4-50ng.d
Type	Cal	Sample	p1 Cal 4-50ng
Acq. Method	AM 28 MDQ P1.m	Operator	Sarah Collins
Sample Position	P2-D11	Comment	
Injection Volume	2		
Acq. Date-Time	12/8/2021 9:54:35 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
a-hydroxyalprazolam	5.692	80005	364.92	72.6	211.97	193362	52.6980 ng/ml
Alprazolam	5.768	272406	1068.83	111.9	3002.16	193362	47.7663 ng/ml

SC

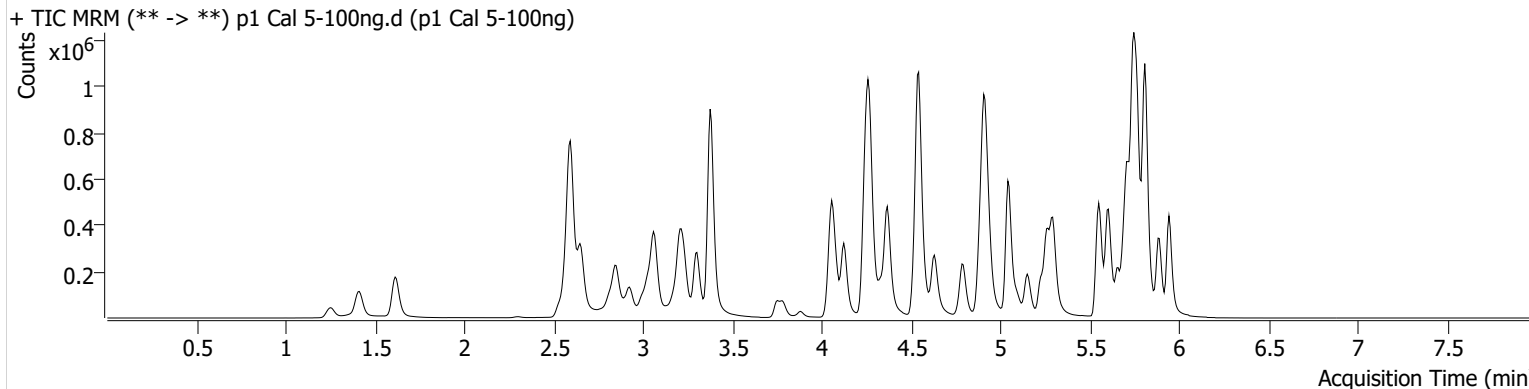


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12082021 AM 28 SC\QuantResults\AM 28 casework.batch.bin
Calibration Last Update 12/8/2021 2:36:22 PM

Instrument	Falco (069901)	Data File	p1 Cal 5-100ng.d
Type	Cal	Sample	p1 Cal 5-100ng
Acq. Method	AM 28 MDQ P1.m	Operator	Sarah Collins
Sample Position	P2-E11	Comment	
Injection Volume	2		
Acq. Date-Time	12/8/2021 10:05:13 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
a-hydroxyalprazolam	5.685	148419	709.08	74.0	917.59	190717	100.3635 ng/ml
Alprazolam	5.768	548570	1436.48	111.0	∞	190717	97.8702 ng/ml

SC

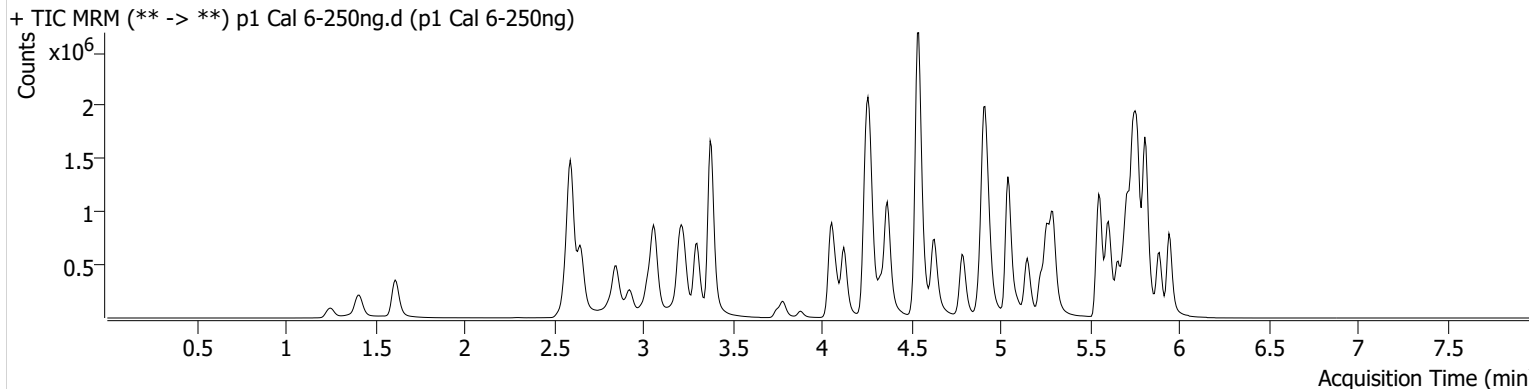


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12082021 AM 28 SC\QuantResults\AM 28 casework.batch.bin
Calibration Last Update 12/8/2021 2:36:22 PM

Instrument	Falco (069901)	Data File	p1 Cal 6-250ng.d
Type	Cal	Sample	p1 Cal 6-250ng
Acq. Method	AM 28 MDQ P1.m	Operator	Sarah Collins
Sample Position	P2-F11	Comment	
Injection Volume	2		
Acq. Date-Time	12/8/2021 10:15:50 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
a-hydroxyalprazolam	5.685	263557	1273.43	73.0	1676.96	152597	224.4661 ng/ml
Alprazolam	5.768	1181061	5050.97	108.8	2222.40	152597	263.9090 ng/ml

SC

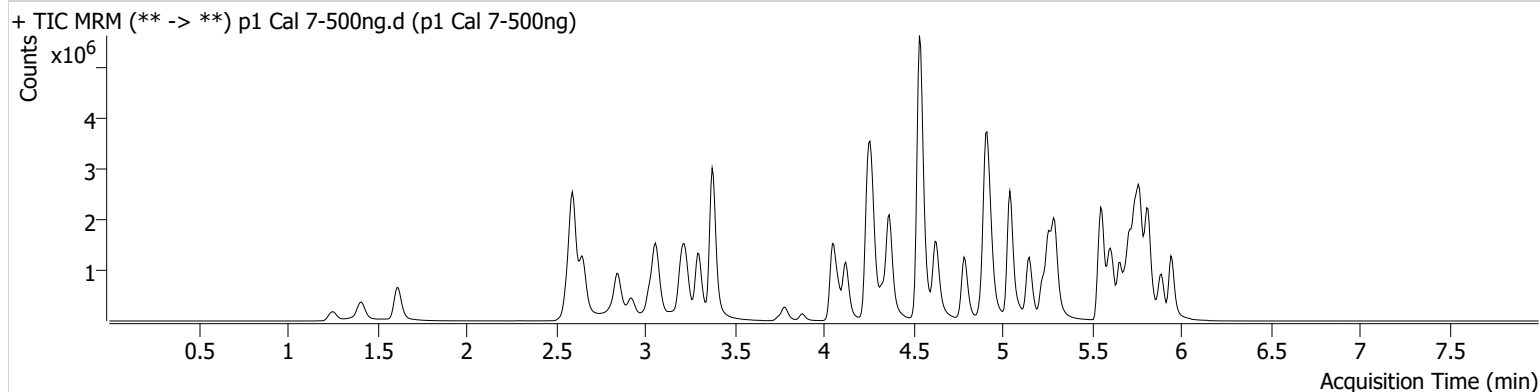


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12082021 AM 28 SC\QuantResults\AM 28 casework.batch.bin
Calibration Last Update 12/8/2021 2:36:22 PM

Instrument	Falco (069901)	Data File	p1 Cal 7-500ng.d
Type	Cal	Sample	p1 Cal 7-500ng
Acq. Method	AM 28 MDQ P1.m	Operator	Sarah Collins
Sample Position	P2-G11	Comment	
Injection Volume	2		
Acq. Date-Time	12/8/2021 10:26:26 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
a-hydroxyalprazolam	5.685	359470	2182.57	73.5	711.91	131839	355.1764 ng/ml
Alprazolam	5.768	2053970	2484.87	109.2	10534.44	131839	531.5602 ng/ml

SC

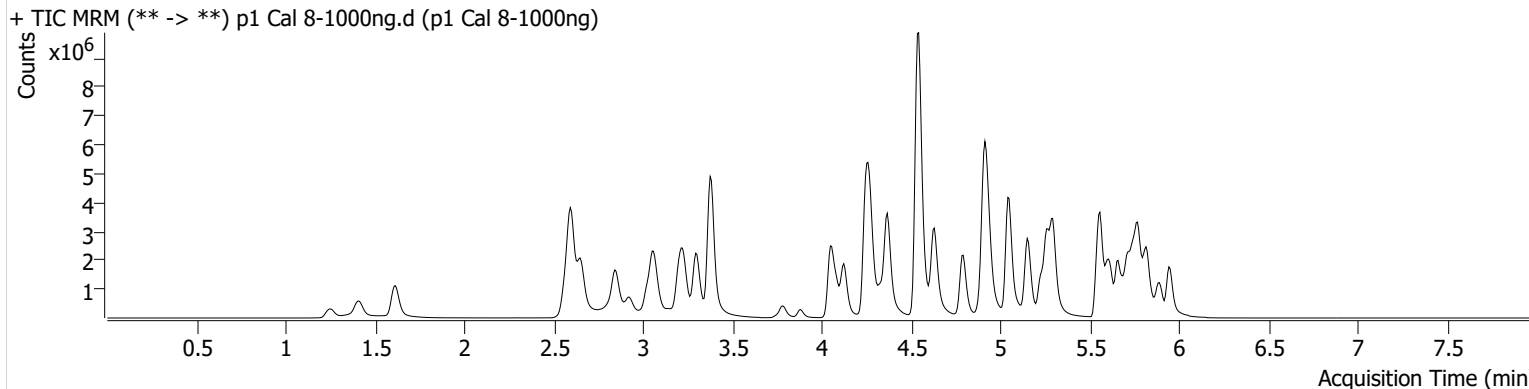


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12082021 AM 28 SC\QuantResults\AM 28 casework.batch.bin
Calibration Last Update 12/8/2021 2:36:22 PM

Instrument	Falco (069901)	Data File	p1 Cal 8-1000ng.d
Type	Cal	Sample	p1 Cal 8-1000ng
Acq. Method	AM 28 MDQ P1.m	Operator	Sarah Collins
Sample Position	P2-H11	Comment	
Injection Volume	2		
Acq. Date-Time	12/8/2021 10:37:02 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
a-hydroxyalprazolam	5.678	421599	525.05	72.2	436.98	101234	543.2431 ng/ml
Alprazolam	5.768	3027518	4690.62	108.3	856.76	101234	1020.6854 ng/ml